When loose ends finally meet

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The tumor suppressor protein 53BP1 decorates DNA damage sites and is instrumental for nonhomologous end joining. Evidence that 53BP1 facilitates synapsis of DNA ends by modulating chromatin dynamics reveals a hitherto unanticipated strategy for joining distant ends.

Faithful transmission of the genetic material requires the concerted action of two principal DNA repair pathways: homology-directed repair and nonhomologous end joining (NHEJ). Whereas a significant length of sequence homology is required for error-free homology-directed repair, NHEJ can proceed with little or no sequence homology and, if not appropriately regulated, can lead to chromosome rearrangements, including translocations. Because of the different requirements for these two repair pathways, NHEJ is predominantly involved in the repair of DNA double-strand breaks (DSBs) when sister chromatids are not available. In addition, NHEJ is pivotal for immunoglobulin V(D)J and class-switch recombinations, where it is instrumental in proper joining of programmed DSBs induced by the RAG1 and RAG2 and AID enzymes.

NHEJ represents a chief strategy for repair of DSBs in mammalian cells. When as little as a single DSB is detected in the genome, several damage-responsive proteins are rapidly recruited to the vicinity of the DNA break to mediate checkpoint activation and DNA repair. One of these, the tumor suppressor protein p53 binding protein-1 (53BP1), accumulates as discrete nuclear foci within minutes at sites of DSB induced by DNA-damaging agents as well as the programmed DNA lesions during V(D)J recombination and class switching. Although previous work has implicated 53BP1 in DNA end joining, the mechanism remains elusive. Using long-range V(D)J recombination and telomere fusions as readouts, two intriguing studies now provide evidence that 53BP1 facilitates distant end joining events through chromatin remodeling[1,2].

53BP1, first identified as a p53 binding protein[3], was ascribed roles in checkpoint control because its depletion in cancer cell lines results in attenuated intra-S and G2/M checkpoint activation[4,5]. Subsequent work suggested that 53BP1 binds to DNA and participates directly in DNA repair, promoting the DNA ligase IV–dependent end joining reaction *in vitro*[6,7]. Consistent with implicated functions in the maintenance of genomic stability, 53BP1-null mice and cells derived from these mice were hypersensitive to DNA-damaging agents and showed chromosome abnormalities typical of failure of proper DNA repair[8,9].

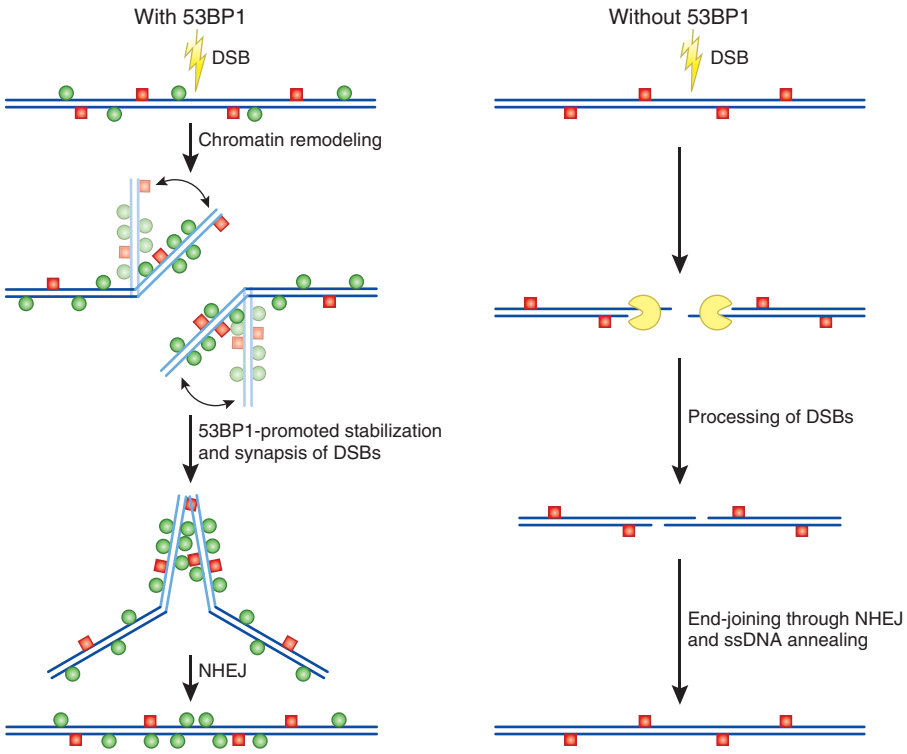

Figure 1 Model for the role of 53BP1 in promoting synapsis of DSBs. 53BP1 (green circles) and other chromatin-bound proteins (red rectangles) reside on the chromatin. Left: induction of DSB results in 53BP1 accumulation at chromatin modified by the damage response, to promote chromatin mobility and synapsis of DSBs through oligomerization of the 53BP1 and/or through its interaction with other chromatin-bound proteins. NHEJ proceeds to restore genomic integrity. Right: in the absence of 53BP1, DSBs are processed by nucleases (yellow). Resection of DNA ends might facilitate alternative repair pathways, including homologous recombination. Subsequent end-joining of DSBs might occur through NHEJ and annealing of single-stranded (ss) DNA, which results in extensive loss of genetic material.

Although mounting evidence implicated 53BP1 in the DNA damage response, it was, paradoxically, not until 53BP1 was found to be essential for class-switch recombination (CSR) that a role in DSB repair was firmly assigned[10,11]. Interestingly, although 53BP1 is critical in CSR, it is only modestly involved in V(D)J recombination[10,11]. These findings were puzzling but suggested that 53BP1 might not directly participate in ligating DNA ends *per se*, but rather would positively regulate NHEJ reactions by an unknown mechanism(s). This idea was finally tested

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by Dimitrova *et al.*[1] by visually assessing the mobility of deprotected telomeres; that is, those lacking the shelterin proteins. Previous studies had already indicated that deprotected telomeres can trigger a cellular response similar to that caused by damage-induced DSBs[12], including 53BP1 accumulation. To support the hypothesis that 53BP1 might be involved in the synapsis of DSBs[10,11], the authors used time-lapse microcopy to reveal that dysfunctional telomeres were more mobile in cells containing functional 53BP1, which correlated with higher frequencies of telomere fusions[1]. The idea that 53BP1 promotes synapsis of DNA ends by increasing local chromatin mobility is particularly attractive, and it agrees with previous reports that described a crucial role of 53BP1 in CSR as opposed to V(D)J recombination, as DNA ends generated during the latter process are relatively close together, hence having little requirement for 53BP1-dependent sampling of DNA ends. This was exactly what Nussenzweig and colleagues[2] found when they reexamined V(D)J recombination proficiency and compared end-joining events between DSBs separated by varying distances. They demonstrated that 53BP1 is specifically required for long-range V(D)J recombination. They also noted extensive degradation of unrepaired coding ends, hence suggesting that 53BP1 might also be involved in stabilizing DSBs before NHEJ. Consequently, the 53BP1 deficiency–associated impairment of long-range V(D)J recombination contributed to increased apoptosis of lymphocytes and lymphopenia.

53BP1 has previously been shown to localize to chromatin through the interaction between its Tudor domain and dimethylated histone residues[13,14]. Mutation of the Tudor domain, which attenuates 53BP1 recruitment to deprotected telomere ends, results in a substantial delay in the fusion of dysfunctional telomeres[1]. Furthermore, depletion of MDC1, an upstream recruiting factor of 53BP1, abrogates the residual NHEJ between telomeres in TRF2 knockout cells in the context of the 53BP1 Tudor-domain mutant. These data clearly illustrate that 53BP1 binding to chromatin is required for optimal NHEJ, at least between distant DNA ends. So how does 53BP1 promote local chromatin mobility? One possible route is through 53BP1 oligomerization, whereby accumulation of 53BP1 at either end of DSBs not only protects the DNA ends from exonuclease activities but can also direct synaptic-complex formation between distant DSBs (**Fig. 1**). In line with this model, a 53BP1 mutant which cannot dimerize was found to be defective in repair[15]. Another, not mutually exclusive alternative would be through other chromatin-bound proteins that directly or indirectly associate with 53BP1. Focal accumulation of 53BP1 might stabilize DSBs and bring these other proteins into close proximity, thus stimulating NHEJ. These 53BP1-associated factors are thus likely to require 53BP1 for their localization to DSBs. Identification of these factors will add to further understanding of how 53BP1 is involved in NHEJ.

Because of the cell cycle–dependent nature of the DNA repair process and the competition between the homology-directed repair and NHEJ repair pathways, it will be interesting to examine NHEJ in nonreplicating cells. As these cells normally do not engage the homology-directed repair pathway, one would be able to better scrutinize how 53BP1 might promote synapsis of DSBs, which is important for genome maintenance. An early study by Lobrich's and Jeggo's groups[16] reported a common DSB repair pathway that requires the ATM kinase, in addition to H2AX, 53BP1, NBS1 and MRE11. What they found was that these proteins are essential for repair of a subset of DSBs, a subset they later showed to be at or near heterochromatin regions[17]. Compiling what we know about 53BP1 function from the literature, the obvious question is how enhanced chromatin dynamics promotes repair of DSBs, in heterochromatin or in general. An elegant study by Ziv *et al.*[18] reported earlier that a change in chromatin dynamics after DNA damage is specifically important for DNA repair, which is at least partially mediated by Ataxia-Telangiectasia Mutated–dependent phosphorylation of the transcriptional co-repressor KAP-1. Is there a direct connection between 53BP1 and a change in chromatin mobility or dynamics after DNA damage? Future studies aiming to address these issues will be of considerable interest.

Over the past few years, we have begun to understand how the cell elicits the DNA damage response to orchestrate processes including checkpoint activation and DNA repair, with the aim of promoting cell survival. During this process, the highly compact structure of the eukaryotic chromatin, which can impede access to the genetic material, must be changed. It has become evident that chromatin regions immediately surrounding DNA breaks are modulated by sundry post-translational modifications, including phosphorylation, acetylation and ubiquitination[19]. In addition, a number of ATP-dependent chromatin remodeling complexes also alter the chromatin structure to facilitate loading of repair proteins and modulate checkpoint responses[20]. The discovery that 53BP1 actively promotes chromatin mobility and synaptic formation between DSBs provides yet another exciting example of the many strategies mammalian cells have evolved to counteract genotoxic stress.

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